

**A Recombinant Immunotoxin Against the Tumor-Associated Antigen Mesothelin
Reengineered for High Activity, Low Off-Target Toxicity, and Reduced Antigenicity**

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Supplementary Figure Legends

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Supplementary Figure S1. Purified recombinant immunotoxins. One microgram of each purified protein, SS1P [1], SS1-LR [2], SS1-LR/GGS [3], SS1-LR/GGS/8M [4], and SS1-LR/GGS R279G [5], was evaluated by SDS-PAGE under reducing (A) and non-reducing (B) conditions. All proteins migrated to the expected molecular weight and were prepared to >95% purity.

Supplementary Figure S2. Relative cytotoxicity *in vitro*. A panel of eight cell lines that express mesothelin was used to compare the cytotoxicities of SS1P (open circles), SS1-LR (open squares), and SS1-LR/GGS (filled squares). Representative individual cytotoxicity assays from data presented in Figure 2 and Supplementary Table 1 are shown.

Supplementary Figure S3. Pharmacokinetics of SS1-LR/GGS/8M in mice. Nude mice were injected intravenously with 10 μ g of either SS1P or SS1-LR/GGS/8M and bled at intervals between 2 and 60 minutes from the time of injection. The serum concentration of immunotoxin at the various intervals was determined by ELISA and fit to a single exponential decay function. The corresponding half-life ($t_{1/2}$) is indicated. Each point is the concentration of immunotoxin in the serum of one mouse.

Supplementary Figure S4. Human antigenicity of SS1-LR/GGS/8M. Neutralizing antibodies produced in patients undergoing treatment with SS1P were evaluated for

reactivity with SS1P (filled circles) and SS1-LR/GGS/8M (filled triangles) using a displacement ELISA. Typical results of the assay are shown here. The concentration of SS1P and SS1-LR/GGS/8M at which binding was inhibited by 50% (IC_{50}) was 356 nM and 345000 nM, respectively for serum from patient #21 (A), and 343 nM and >4330000 nM for serum from patient #36 (B). The IC_{50} ratios of SS1P to SS1-LR/GGS/8M are 0.10 % (A) and <0.0079 % (B). Sera from a total of five patients were analyzed by this method, and the IC_{50} ratios between SS1P and SS1-LR/GGS/8M are shown in Figure 5.